Quantitative Trait Loci (QTL) Mapping of Drought Tolerance at Seedling Stage of Rice (*Oryza Sativa*)

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Abstract
Drought tolerance is a quantitative trait. Quantitative trait loci (QTL) analysis was carried out for drought tolerance at seedling stage to determine the region of the genome of rice responsible for drought tolerance. One hundred and nine (109) F_2 and F_3 progenies of the cross between Eh la Chiu (drought susceptible variety) and Ma Hae (drought tolerant variety) were used in the QTL analysis. The F_2 progenies were genotyped using rice Simple Sequence Repeat (SSR) markers at the IITA Central Molecular Biology Laboratory. The F_3 lines were subjected to drought stress under upland field conditions during the dry season of 2017 at the IITA field, Ibadan, Nigeria. Continuous variation and transgressive segregation were observed in leaf drying symptoms and drought recovery among the F_3 lines screened for drought tolerance. Only 38 of the 495 SSR markers genotyped were polymorphic between the parents but 19 polymorphic SSR markers were used to genotype the F_2 individuals. Only marker RM 585 with Logarithm of Odd (LOD) at 2.5 showed a significant relationship to drought tolerance. Therefore RM 585 could be used in selecting for drought tolerant rice lines.

Keywords: Rice, Drought tolerance, Polymorphism, Quantitative Trait Loci, SSR markers, Transgressive segregation

Introduction

Rice is one of the most important foods consumed by over 50% of the world's population mainly in Asia and sub-Saharan Africa (Akpokodje *et al*., 2001). It ranks third after wheat and maize in production (Guimara˜es, 2009; Ajah and Ajah, 2014). Rice provides 21% and 15% global human per capita energy and protein, respectively (FAOSTAT, 2001). Human consumption accounts for 85% of the total production of rice, compared to 72% for wheat and 19% for maize (Rice Almanac 2013). Protein content in rice is 8-9% while wheat has 11-12% (Khush, 1997). Rice is a major source of calories for the urban poor. Rice is a major staple food crop in West Africa. Although its production increased up to 170% between the 1970s and the early 2000s, consumer demand is still more than production (Africa Rice Centre, 2008) as only 40% of current rice consumption is satisfied by domestic production in the region. In several African states, rice availability and rice prices have become a major determinant of the welfare of the poorest segments of consumers who are least food secure. Rice is, therefore, one of the most important commercial food crops

of the world (IRRI, 2005).

Drought is one of the major limitations to food production worldwide and is endemic particularly in the semi-arid tropics. Improving drought tolerance and productivity is one of the most difficult tasks for cereal breeders (Vinod *et al*., 2006). The total rain-fed rice area accounts for about 85 % of the total rice lands in Sub-Saharan Africa (Ikeda, 2004). Lower and irregular rains considerably increase the potential for intra-seasonal drought spells and upland rice yields are generally low and further destabilized by frequent drought (Lawson and Alluri, 1986). With global changes in environmental conditions, and the need to develop resilience in poor farming communities, efforts need to be made to improve rice varieties that can withstand these environmental changes.

Methods of improving rice include conventional breeding such as pedigree, backcross, mass selection, etc. and biotechnological approaches using molecular markers such as Simple Sequence Repeats (SSRs), Restriction Fragment Length Polymorphism (RFLP), Random Amplification Polymorphic DNA (RAPD) etc. has become useful tools for the study of genetic relationships (Garcia-Mas *et al*., 2000). Mapping of quantitative trait loci (QTLs) provides precious information for establishing breeding programs. Materials such as recombinant inbreds, doubled haploids and backcross families are used for mapping QTLs under different environmental conditions. (Yu *et al*, 1991).

To develop rice varieties adaptable to drought conditions, there is a need to analyze the genetic traits of rice responsible for drought tolerance. Therefore, the objective of this study was to map the QTL of drought tolerance at the seedling stage of rice under upland field conditions.

Materials and Methods

Experimental location

The experiment was carried out in an upland ecology at the Africa Rice Center, Nigeria station, International Institute of Tropical Agriculture, Ibadan (latitude 7° 30'N and longitude 3° 45'E).

Source of Experimental Materials

The rice genotypes used in this study were 109 lines of F_2 and F_3 progenies of the cross between two varieties Eh Ia Chiu and Ma Hae. Variety Eh Ia Chiu is drought-susceptible from Taiwan while Variety Ma Hae is drought-tolerant from Thailand.

Experimental Procedures

Phenotyping of the rice lines for leaf drying

One hundred seeds were sown using drilling method of planting during the dry season (January to March) of 2017 directly in 30 cm long rows, augmented experimental design was used, all agronomic practices such as land clearing, ploughing and harrowing was carried out. Fertilizer, NPK 15:15:15 was applied at the rate of 40 kg/ ha after planting. Watering was carried out for 14 Days After Sowing (DAS) then stopped at 15 DAS onwards. No rainfall was observed during the period of drought stress.

The seedlings were observed and scored for their response to drought according to IRRI standard evaluation system (IRRI,2013) on a scale of 0 - 9 (Plate 1) where:

0 - No symptoms.
1 - Slight leaf tip drying.
3 – Leaf tip drying extended up to ¼ length in most leaves.
5 - 1/4 to1/2 of all leaves fully dried.
7 - More than 2/3 of all leaves fully dried.
9 - All plants apparently dead.

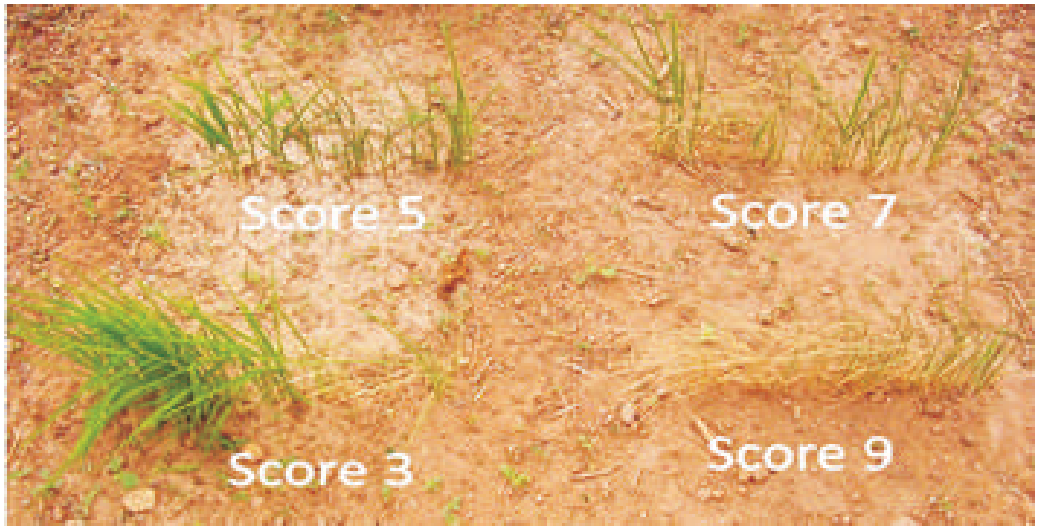

Plate 1: Response of the F_3 lines to drought depicting the different response scales

Genotyping

Leaf tissue of each genotype of the F_2 lines used for the genotyping were collected from the field and placed on ice prior to transport to the laboratory for total genomic DNA extraction.

Extraction of DNA

Total genomic DNA was isolated from fresh leaf tissues, using the modified Dellaporta method of DNA extraction (Dellaporta *et al*., 1983). The concentration of the DNA was quantified using a spectrophotometer at a wavelength of 260 to 280 nm. The readings at A260 x 50 (a constant) x dilution factor (2:500, i.e. 250) was used to calculate the concentration of each sample. The quality of the DNA was checked on 0.8% agarose gel and each sample was diluted to 25 ng/l.

Primer screening

A total of 495 SSR primers were screened and the two parental lines were used to determine the polymorphic primers to be used for the study. A master-mix was made for each sample of DNA in a 7.1 L reaction volume as follows: 3.0 L milli-q H_2O, 1.0 L 10X buffer, 0.2 L dNTPs (10mM), 0.3 L forward and reverse primer (20 pmol), 0.1 L Taq polymerase (5u/L) and 2.5 L genomic DNA. The PCR amplification temperature programme used was as follows: 94 ºC for 2 minutes (1 cycle); 94 ºC for 30 seconds, 55 ºC for 30 seconds, 72 ºC for 30 seconds (34 cycles); 72 ºC for 2 minutes (1 cycle) and 15 ºC touchdown. PCR amplification of products was checked on 2% agarose gel electrophoresis in 0.5X TBE buffer.

Polymorphism survey

This was conducted using the parental lines. Gel electrophoresis of PCR amplification products was performed using 2% agarose gel in 0.5X TBE buffer. The electrophoresis was run at 100 volts for 1 hour 40 minutes and the gel was stained in ethidium bromide solution for 3 - 5 minutes. The gel was then rinsed in fresh distilled water for 10 - 15 minutes and visualized under ultraviolet light to view amplification of the bands. The photograph of the gel image was taken thereafter using a gel documentation system.

Primers that can discriminate between the parents were selected as polymorphic, while monomorphic primers are those that could not discriminate between the parents. Fragment sizes were estimated from linear interpolation by means of a DNA size standard (1KB plus ladder.). All the 12 rice chromosomes were represented to cover the rice genome and the location of the marker on the chromosome was also noted (one marker was selected to represent two or more markers that were very close to each other on the chromosome).

Scoring of bands from the gel electrophoresis

The bands from the gel electrophoresis were scored on a scale of 1 -3 where 1 = same band size as the first parent, P1, 2 = same band size as the second parent, P2, 3 = heterozygous and 0 -= missing data. The QTL analysis was performed by Qgene version 4.0 using both the phenotyping and genotyping data taking the Logarithm of Odd (LOD) at 2.5.

Drought recovery

The F_3 lines were observed and scored for their recovery from drought stress according to IRRI standard evaluation system (IRRI, 2013) on a scale of 1 - 9 at the onset of rainfall as from March, 2017 through June, 2017* where

1 – 90-100% recovery
3 – 70-89% recovery
5 – 40-69% recovery
7 – 20-39% recovery
9 – 0-19% recovery

□ ***Appendix 1.***

Results

The frequency distributions of the lines for leaf drying at different time intervals after the commencement of drought stress are shown in Figure 1. At 16 DAS (i.e. 2 days after commencement of water stress) all the progeny lines and the parents had a score 0, implying that none showed symptoms of drought stress at the onset of the experiment. By 23 DAS, 6 lines scored 0, 58 lines scored 1, 36 lines scored 3 while 9 lines scored 5. However, both parental lines scored 0. At 35 DAS, no line scored 0, 2 lines scored 1, 38 lines scored 3, 56 lines scored 5 while 13 lines scored 7. And both parental lines scored of 3. At 48 DAS, no line scored 0 or 1. This shows an increasing effect of drought among the lines. The drought tolerant parent scored 5 while the drought susceptible parent scored 7. Among the progenies, 12 lines scored 3, 54 lines scored 5, 42 lines scored 7 while 1 line scored 9. At 60 DAS, no line scored 0, 1 and 3, 18 lines scored 5, 66 lines scored 7 while 25 lines including both parental lines scored 9.

Of 495 rice SSR markers screened, 38 yielded polymorphism. The 19 markers selected were then used to genotype the F_2 individuals. Polymorphic markers were selected across the 12 chromosomes of rice to represent rice genome. Table 1 shows the list of markers, chromosome number and the location on the genome.

Plate 2 shows the segregation of F_2 population using RM 224 marker, M is the molecular size marker, P_1 is Eh la chiu while P_2 is Ma Hae. The band size of F_2 individual revealed the same size as P_1, P_2 or having both (heterozygous). Out of the 19 SSR markers used for the genotyping of F_2 individuals, only RM 585 gave a significant relationship to drought tolerance when QTL analysis was carried out taking the LOD at 2.5 (Table 2).

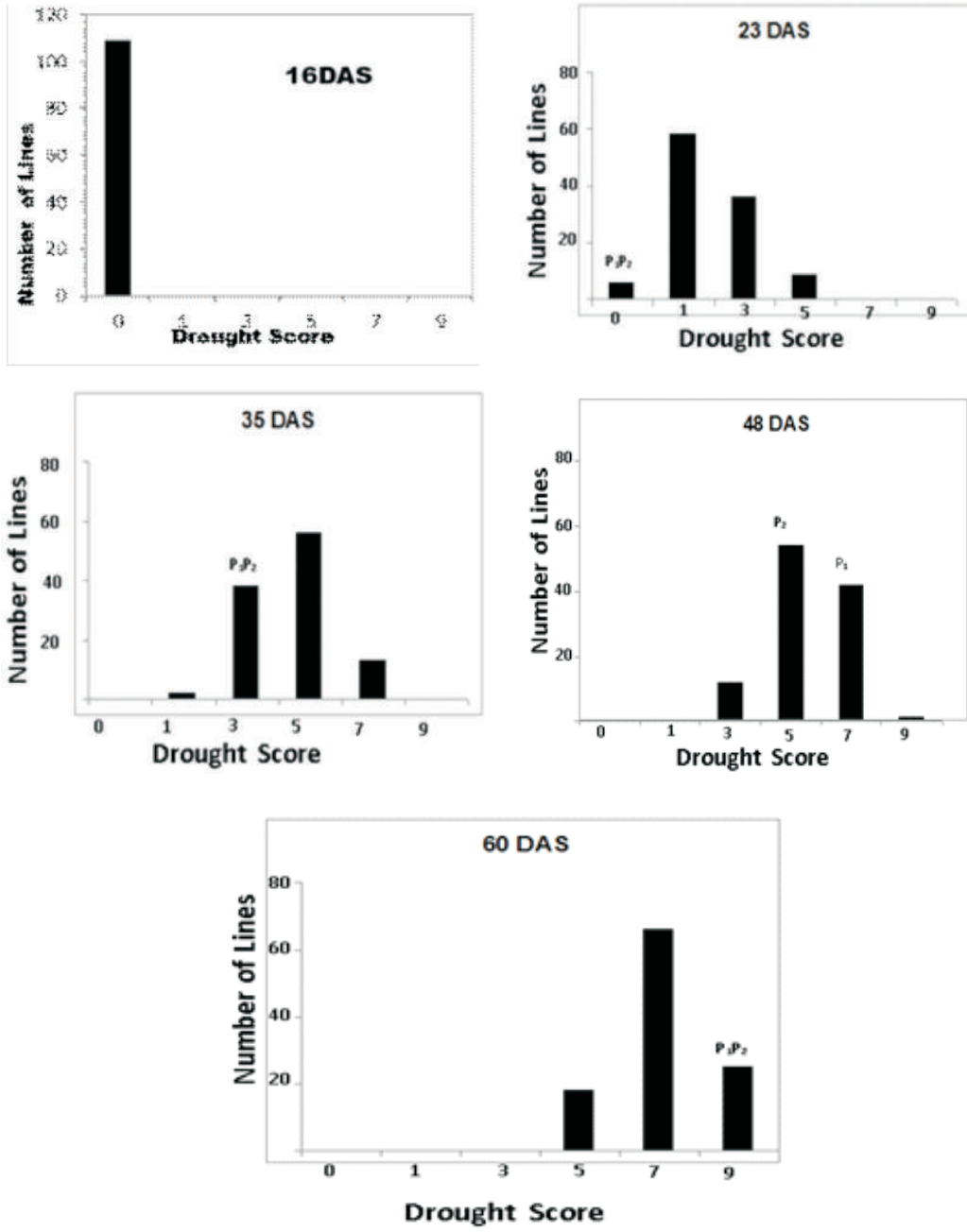

Figure 1: Frequency Distribution of the F_3 Lines for Leaf Drying

P_1 – Eh la Chiu ; P_2 – Ma Hae

Table 1: Polymorphic Markers used in genotyping F_2 lines

Marker	Chromosome No.	Location on chromosome (cM)
RM 220	1	28.4
RM 263	2	127.3
RM 561	2	74.1
RM 570	3	221.1
RM 426	3	157.3
RM 317	4	118.3
RM 241	4	106.2
RM 249	5	65.8
RM 480	5	130.6
RM 412	6	142.4
RM 585	6	25.1
RM 481	7	3.2
RM 432	7	43.5
RM 281	8	128.6
RM 285	9	1.8
RM 467	10	46.8
RM 206	11	102.9
RM 224	11	120.1
RM 17	12	109.1

(Temnykh, *et al*., 2001)

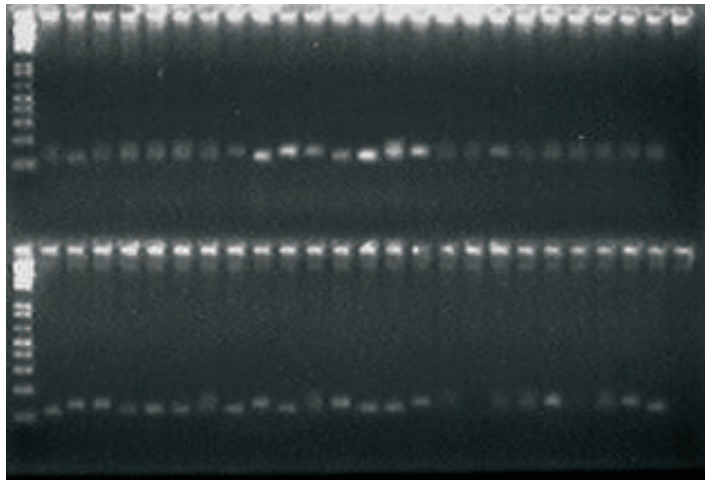

Plate 2: Segregation of RM 224 (SSR Marker) in the F_2 population of progeny lines from the cross between Eh la Chiu and Ma Hae.
M - Molecular size marker; P_1 - Eh la Chiu; P_2 - Ma Hae

Table 2: Quantitative Trait Analysis of F_2 Population for Drought Tolerance at Seedling Stage

Trait	Marker	LOD	Additive effect	Dominance effect
DS 14	RM 585	2.54	0.286	0.136
DS 16	RM 585	2.61	0.286	0.155
DS 18	RM 585	2.74	0.219	0.305
DS 19	RM 585	2.71	0.248	0.205

LOD - Logarithm of odd

DS 14 - Drought score at 48 DAS; DS 16 - Drought score at 51 DAS; DS 18 - Drought score at 53 DAS; DS 19 - Drought score at 55 DAS

Recovery - Response of F_3 lines after drought stress during raining season

Figure 2 shows the frequency distribution of F_3 lines that recovered from drought stress during the raining season of the same year 2017. The F_3 lines response to drought recovery at the onset of raining season revealed that both Parent 1 and Parent 2 did not recover from the drought stress while at 122 DAS only two F_3 lines had score 1, 11 had score 3, 18 lines had score 5, 54 lines had score 7 and 24 lines had score 9 (Figure 2). Whereas at 150 DAS, only two F_3 lines had score 1, 11lines had score 3, 12 lines had score 5, 52 lines had score 7 and 32 lines had score 9. Plate 3 shows the response of the F_3 lines at 60 DAS and 122 DAS respectively.

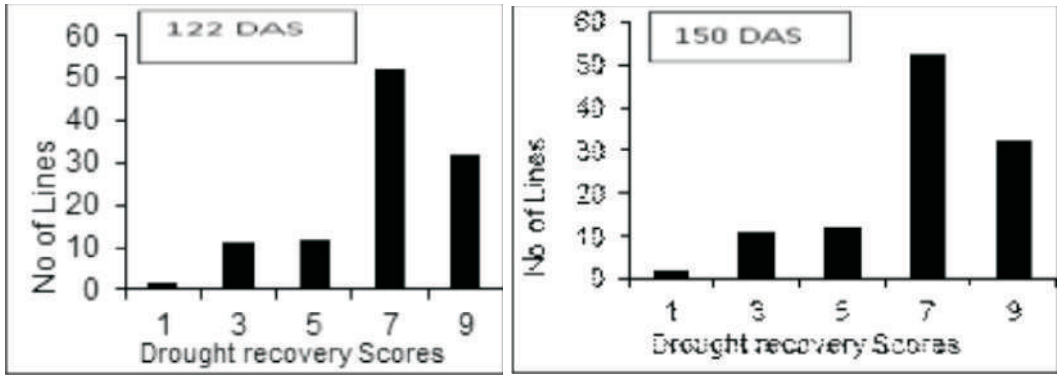

Figure 2: Frequency distribution of F_3 lines that recovered after drought stress during raining season (122-150 DAS)

Discussion

Continuous segregation was observed for leaf drying in the evaluation of the F_3 lines of the cross between Eh la Chiu and Ma Hae under upland field conditions. The F_3 lines progressed from good drought score (0-3) to worst drought score (5-9) as the number of days of drought stress increased. While it was expected that the parents will set the boundaries for the drought tolerance score, some of the progenies had a better drought score than the parents and this indicates transgressive segregation for drought tolerance among the F_3 progenies. This indicates good prospects of selecting superior genotypes for drought tolerance among segregating populations.

QTL analysis is based on the principle of detecting an association between phenotype and the markers. Markers are used to partition the mapping population into different genotypic groups based on the

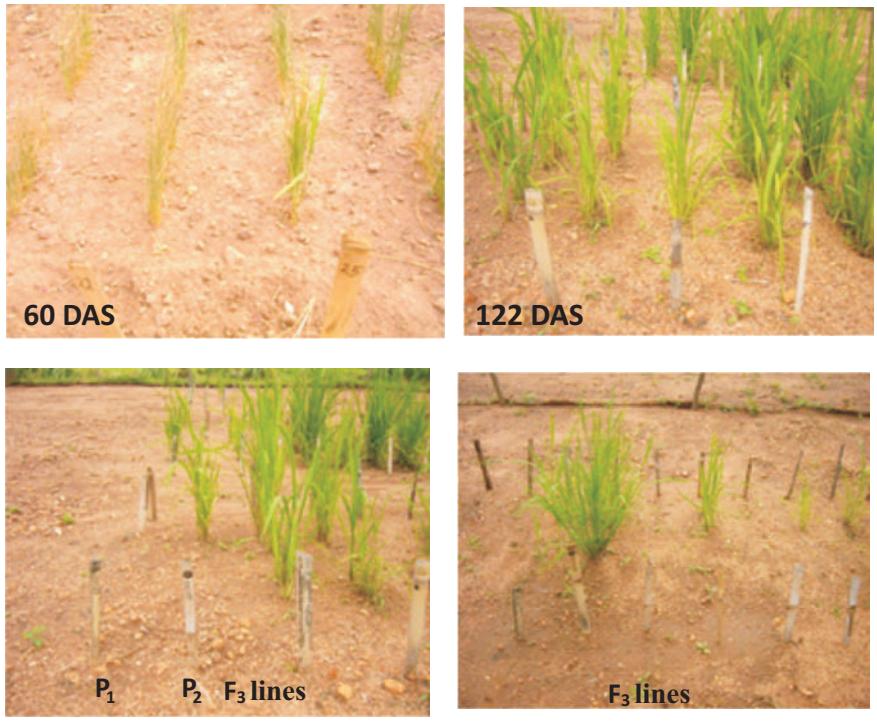

Plate 3: Recovery of F_3 lines during raining season at 120-150 days after sowing

presence or absence of a particular marker locus and to determine whether significant differences existed between groups with respect to the trait being measured (Tanskley, 1993). In genotyping the F_2 individuals, only RM 585 out of the 19 SSR markers used showed a significant relationship to drought tolerance and it is located on chromosome 6 at 25.1cM. At 53 DAS, RM 585 was tightly linked to a QTL controlling drought tolerance. Therefore, the QTL and the marker could be jointly inherited together in the progeny and drought could be tolerated up to 55 DAS.

The remaining 18 markers that showed no significant relationship to drought tolerance indicate that the markers may be loosely linked or unlinked to a QTL and will be randomly inherited with the QTL. The results of this study agrees with the report of Temnykl *et al*., (2001) that a QTL for drought is located on chromosome 6 at 25.1cM. A candidate gene for drought tolerance (CRTDRE) located on chromosome 6 at 12.9 cM was also reported by Vinod *et al*., (2006). This could therefore be used for selecting drought tolerant rice lines, as it may greatly increase the efficiency and effectiveness in plant breeding methods. Once markers that are tightly linked to genes or QTLs of interest have been identified prior to field evaluation of large numbers of plants, breeders can use specific DNA marker alleles as a diagnostic tool to identify plants carrying the genes or QTLs of interest. This will save considerable time and resources in conducting field trials at particular times of the year or at specific location. This will also enhance selection at seedling stage since genotype can be determined as from 7 days

after sowing. The advent of saturated molecular maps promises rapid progress towards the improvement of crops for genetically complex traits like drought resistance via analysis of QTL (Adam *et al*., 2002).

The recovery response of F_3 lines after drought stress during raining season revealed continuous and transgressive segregation among the F_3 lines in recovery ability from drought stress while both Parent 1 and Parent 2 did not recover from the drought stress. The ability to recover after a period of drought is a very important trait to acquire in new rice lines given the trend in delayed rainfall and occurrence of periods of drought within the rainy season. This recovery, in rice lines will support resilience in poor communities where rice is important for their livelihood.

Conclusion

Drought resistance is controlled by quantitative trait loci (QTL) RM 585 located on chromosome 6 and drought tolerance scores at 48 DAS, 51 DAS, 53 DAS and 55 DAS showed a significant relationship to drought tolerance using the cross between Eh la Chiu and Ma Hae under upland field conditions. Further work is recommended for the fine genetic mapping of drought tolerance, QTL for yield under drought condition and QTL for recovery.

Acknowledgement

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***Appendix 1.**

***Monthly Means of Weather data at IITA, Ibadan, Nigeria from January to June 2017**

Month	Rainfall	Solar Radiation	Minimum Temperature	Maximum Temperature	Minimum Relative Humidity	Maximum Relative Humidity
	(mm)	(MJ/m²/day)	(°C)	(°C)	(%)	(%)
Jan.	0.0	14.6	21.4	33.6	25.7	89.8
Feb.	0.0	13.8	22.9	35.2	22.8	84.7
Mar.	2.1	16.7	24.0	34.8	32.7	86.8
Apr.	3.5	16.3	23.2	33.0	49.7	87.3
May	6.1	16.5	22.8	31.0	55.3	86.9
Jun.	11.7	14.7	22.9	30.4	62.6	91.3

Source: IITA Ibadan weather station, 2017